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Comparison of serum IL-1beta and C-reactive protein levels in early diagnosis and management of neonatal sepsis

Confronto tra i livelli sierici di IL-1 beta e proteina C reattiva nella diagnosi precoce e nella gestione della sepsi neonatale

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INTRODUCTION

Septicaemia is a critical disease in the neonatal period and during infancy [1-3]. Bacterial infections in infants (particularly preterm infants) may immediately lead to generalised sepsis, and if not treated accordingly, may lead to high rates of morbidity and mortality. This disease has non-specific signs and symptoms that must be treated properly and quickly; otherwise, they can disrupt the clinical status of patients and become life-threatening [4].

The incidence in some countries is low (1.4%-3.2%), although early onset and late onset septicaemia have different incidences but other factors such as gestational age play a major role in the incidence of septicaemia, which increases with decreasing gestational age [1-3]. In addition, the case fatality rate for early and late onset sepsis has been reported as high as 40% and 19.7%, respectively [5]. According to a report of the World Health Organization, about five million deaths are caused annually by neonatal septicaemia [6].

Early diagnosis of sepsis in neonates and infants presents a clinical dilemma. A common method is to be guided by blood culture results after 48 to 72 hours of incubation, by which time 98% of cultures ultimately yielding an organism will be positive. However, initiation of antibiotic therapy before obtaining the diagnostic results is recommended for neonates and infants with clinical signs or epidemiological factors associated with sepsis.

Previous studies reported low sensitivity for white blood cell (WBC) counts in the diagnosis of infections in infants. Moreover, the combination of some paraclinical tests such as total neutrophil count, ratio of immature neutrophils to total neutrophil count, and platelet count are not adequately sensitive or specific for the diagnosis of sepsis in infants, although another study showed that two serial normal immature to total neutrophil ratio accompanied with negative blood culture could be indicative of a non-infected neonate [7-9, 3].

C-reactive protein (CRP) is an acute-phase pro-

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tein found in the blood, whose levels rise in response to inflammation within six to eight hours [10]. CRP with a half life of 19 hours may increase more than 1000 fold following an infection [11]. In some studies, CRP levels increased in septic infants [12, 13]. However, another study indicated that measuring CRP levels is not efficient for the early diagnosis of infections in infants, since they are useful for confirmation or rejection of an infection 24 hours after infection is suspected [14].

Sepsis and endotoxins activate monocytes, macrophages, lymphocytes, fibroblasts and endothelial cells. These activated cells produce and secrete substances such as interleukin-1(IL-1), tumour necrosis factor-α (TNF-α), interferon-α, IL-6, IL-8, and other proinflammatory cytokines. IL-1β is a member of the interleukin 1 cytokine family. This cytokine is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE). This cytokine is an important mediator of the inflammatory response. Clinical signs and symptoms in neonatal sepsis are non-specific, and in addition laboratory parameters such as WBC count, erythrocyte sedimentation rate and CRP levels are of limited diagnostic value, and standard diagnostic procedures may delay treatment and result in poor prognosis [7, 15-19].

There is still no satisfactory test capable of early and rapid diagnosis of sepsis in infants. Therefore the aim of this study was to compare serum IL-1beta and C-reactive protein levels in early diagnosis and management of neonatal sepsis.

PATIENTS AND METHODS

In this cross-sectional study, blood samples were obtained from 83 infants hospitalised in a children's hospital in Qazvin in whom infection was suspected. This study was approved by the ethics committee of Qazvin University of Medical Sciences and all parents completed the informed consent form.

After the infants were admitted and examined by paediatricians and subspecialists, routine tests for sepsis were arranged and blood samples were collected. The infants that presented with more than three of the following signs and symptoms of sepsis were screened for sepsis:

- maternal risk factors such as maternal fever, alcohol consumption, premature rupture of membranes (more than 24 hours), chorioamnionitis, and maternal urinary tract infection [20];
- neonatal risk factors such as low birth weight (less than 2500 g) and premature birth (less than 37 weeks) [20];
- signs and symptoms of sepsis, including anorexia, lethargy, temperature instability (fever and hypothermia), jaundice, apnea, respiratory distress, tachycardia (>180/min), tachypnea (>60/min), cyanosis, and vomiting [4, 19, 20].

To perform serum IL-1β and quantitative CRP tests, blood samples were placed in ice containers and delivered to the laboratory. The serum was removed from the blood samples at 4°C and kept in refrigerators at -30°C until the time of testing.

A mini nephelometric device (The Binding Site Company, Birmingham, UK) was used to measure CRP. All samples were diluted with the nephelometry dilution solution at a 1/40 ratio. Twenty microlitres of each serum sample were poured into a cuvette and placed in the cuvette chamber of the nephelometer. Then 400 µL of CRP buffer and 40 µL of CRP reagent were added to the cuvette, which was immediately placed inside the nephelometer. In this reaction, the CRP present in the serum binds to polystyrene microparticles and forms an antigen-antibody complex; these resultant immune complexes are exposed to the laser beam of the device.

The scattering intensity of the laser beam measured by the device is proportional to the concentration of the CRP in the samples. A serum CRP level >10 mg/L was considered abnormal. IL-1β levels were measured by enzyme-linked immunosorbent assay (ELISA). Microplates were covered with monoclonal antibodies against IL-1β. First, 50 µL of serum from each patient and 50 µL of assay buffer were added to each well. Serial dilutions were made from the standard solution and added to the wells. A 50 µL-volume of monoclonal IL-1β conjugated to biotin was added to each well. After 2-hr incubation at room temperature and 4 rinses, 100 µL of streptavidin horseradish peroxidase (HRP) was added to the wells. Following 1-hr incubation at room temperature and 4 rinses, 100 µL of substrate solution was added to the wells. After 15 min, the reaction was stopped using stopping solution and the contents of the plates were read at 450 and 620 nm with an ELISA plate reader. The concentration of IL-1β was

calculated by drawing the standard curve.
Phase I culture medium D was used to perform the blood culture. The culture medium was added to 1-3 mL of blood and the cultures were placed into an incubator for 5 days at 35°C. The cultures were examined daily for bacterial growth. If bacterial growth was observed, selective media were used to identify the type of bacterium present in the cultures. Urine and cerebrospinal fluid (CSF) cultures were also performed for all subjects.
Gold standard criteria for diagnosis of sepsis were as follows:

- documentation of a positive culture from blood, CSF or urine [21];
- clinical signs of sepsis with two or more of the following para-clinical results: WBC count <4000 or >10000 per mm^3, band cells to total neutrophil count ratio >0.2 and a positive acute phase reactant test [4].

The collected data were analysed by independent sample T-test. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for the IL-1β and CRP in comparison to the gold standard.

RESULTS

In this study, samples from 83 infants (41 girls and 42 boys) were examined. There were 12 positive blood cultures, 1 positive cerebrospinal fluid culture, and 3 positive urine cultures among the 83 samples (Table 1). The serum samples were tested to measure IL-1β by ELISA. The horizontal line that was drawn for IL-1β was equal to the cutoff value of 3.72 pg/mL.

Table 1 - Characteristics of the positive cultures.

Sample	*Type of microorganisms*	*Number*
Blood	*Klebsiella* spp	4
	Coagulase negative staphylococci	3
	Escherichia coli	2
	Staphylococcus epidermidis	1
	Coagulase positive staphylococci	1
Urine	*Klebsiella* spp	2
	Escherichia coli	1
Cerebrospinal fluid	*Escherichia coli*	1

Among the 83 patients, 60 patients showed negative results for IL-1β and 23 patients showed positive IL-1β results. The sensitivity, specificity, PPV and NPV of the IL-1β ELISA for diagnosis of sepsis were 27%, 71%, 25% and 73%, respectively. The sensitivity, specificity, PPV and NPV of the IL-1β for diagnosis of positive blood culture were 33%, 74%, 18% and 86%, respectively.
CRP was measured upon patient admission prior to the initiation of treatment. The horizontal line that was drawn for CRP was equal to the cutoff value of 10 mg/L. Serum CRP levels <10 mg/L were considered negative and those >10 mg/L were considered positive. The negative and positive CRP rates of the patients were 26 and 57, respectively. The sensitivity, specificity, PPV, and NPV of the serum quantitative CRP for diagnosis of sepsis were 76%, 60%, 40%, and 88%, respectively.

DISCUSSION

Microbial infection remains a major cause of infant death. Given that prognosis of sepsis in infants largely depends on an immediate diagnosis and appropriate antibiotic treatment, patients undergo extensive laboratory testing and empirical antibiotic treatment. The final diagnosis of sepsis is confirmed by blood culture, which lasts at least 48-72 hr and shows positive results in 30-70% cases. Furthermore, advanced blood testing may not be available in all health centres [4].
Numerous studies have been conducted using laboratory tests for sepsis; however, there is still no satisfactory test capable of accurately diagnosing sepsis in infants [4, 13, 18, 22-25]. Due to the importance of immediate diagnosis of sepsis, haematological parameters have also been examined. Most researchers conclude that examining haematological parameters in septic infants with negative blood cultures may be useful [25, 26]. The study conducted by Shirazi et al. reported that the sensitivity and specificity of haematological parameters were 60% and 70%, respectively.
A study by Kurt et al. of haematological parameters found that the sensitivity and specificity of absolute neutrophil count were 71.4% and 88%, respectively [4]. Over the last few decades, it has been reported that production of various mediators of inflammation increases during sepsis. Studies of cytokines such as IL-1β and

TNF-α, which are produced during the initiation of inflammatory cascade, have yielded differing results. In the study by Kurt et al., serum levels of IL-1β and TNF-α in septic infants increased considerably.
However, other studies have reported results that were contrary to those of the present study [27- 30]. Another study by Atici et al. found that serum IL-1β levels decreased in septic infants. Furthermore, serum IL-1β levels were not dependent on whether the infection was caused by gram-negative or gram-positive microorganisms.
The authors concluded that serum IL-1β levels decreased both in preterm and term infants and that the gestational age was inconsequential [31]. The results of the above study were also inconsistent with those of the present study. In a separate study reported by Ucar et al., serum levels of IL-1β in septic infants were also low, contrary to the findings of the present study [32]. Interestingly, it appears that the monocytes of infants are unable to secrete sufficient IL-1β and prostaglandin E2, and IL-6 may also suppress secretion of IL-1β and TNF-α in infants [22, 29-33]. Similar to the present study, quantitative analysis of CRP as an acute phase reactant by Ucar et al. had a rather high sensitivity and specificity [32].

Utilization of quantitative instead of qualitative CRP and comparing its results with Il-1β is the strength of this study. The limitations of the present study are the cross sectional design and the small population size.
Based on the results of the present study and other studies reported in the literature, quantitative serum CRP measurement could be a suitable test to diagnose sepsis in infants. Moreover, the advantage of this approach lies in the reduction in the number of infant ward admissions as well as a reduced number of admission days. Conducting similar experiments in developing countries in which hospital facilities and resources are limited might be quite advantageous.

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Keywords: Il-1Beta, CRP, sepsis, newborn, diagnosis.

SUMMARY

Clinical signs and symptoms of non-infectious diseases are similar to those of infectious diseases during infancy.
Therefore, rapid new methods for diagnosis of infections in infants are urgently needed. To examine the utility of measuring serum IL-1β for immediate diagnosis of sepsis in infants, in this cross-sectional epidemiological study blood samples were taken from 83 infants (41 female and 42 male) in whom infection was suspected and who were admitted to hospital.
To perform serum interleukin (IL)-1β and quantitative C-reactive protein (CRP) tests, blood samples were placed in ice containers and delivered to the laboratory. The serum was removed from the samples at 4°C and stored in refrigerators at −30°C until the time of testing.
The results were analysed by t tests. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of serum IL-1β were 27%, 71%, 25%, and 73%, respectively. Sensitivity, specificity, PPV and NPV of serum quantitative CRP were 76%, 60%, 40% and 88%, respectively.
Hence quantitative serum CRP measurement for the diagnosis of infections is more efficient than measuring serum IL-1β levels. In general, quantitative serum CRP measurement as an alternative to other tests such as serum IL-1β assays might be an ideal test for diagnosing infections in infants.

RIASSUNTO

La similitudine che si riscontra, nell'età infantile, tra segni clinici e sintomi delle malattie infettive e non infettive rende necessario poter disporre di nuovi e rapidi metodi per la diagnosi di infezione nel neonato.
Nell'intento di valutare l'utilità della determinazione dei livelli sierici di interleuchina (IL)-1β per la diagnosi immediata di sepsi nel neonato, in questo studio epidemiologico cross-sezionale sono stati raccolti campioni di sangue da 83 neonati (41 femmine e 42 maschi) ricoverati in ospedale per sospetto di infezione. Per effettuare i test di rilevazione della IL-1β sierica e della proteina C reattiva (PCR), i campioni di sangue sono stati posti in contenitori con ghiaccio e inviati al laboratorio dove il siero di ciascun campione è stato congelato a -30°C fino al momento del test. I risultati sono stati analizzati con il t test. Sensibilità, specificità, valore predittivo positivo (PPV), e valore predittivo negativo (NPV) relativi alla determinazione della IL-1β sierica sono risultati pari a 27%, 71%, 25%, e 73%, rispettivamente. Sensibilità, specificità, PPV e NPV relativi alla determinazione quantitativa della PCR sierica sono risultati pari a 76%, 60%, 40% e 88%, rispettivamente. Per la diagnosi di infezione, dunque, la misura quantitativa della PCR è più efficiente rispetto a quella della IL-1β. In linea generale, la determinazione quantitativa della PCR nel siero in alternativa ad altri test, quale la determinazione della IL-1β sierica, potrebbe costituire un test ottimale per la diagnosi di infezione nel neonato.

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